

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

Claim 1 (Currently amended): An apparatus for determining a threshold cycle number in a nucleic acid amplification reaction, the apparatus comprising:

- a) a thermal cycler suitable for a nucleic acid amplification reaction;
- b) at least one detection mechanism for measuring, at a plurality of different times during the an amplification reaction conducted using the thermal cycler, at least one signal whose intensity is related to the quantity of a nucleic acid sequence being amplified in the reaction; and
- ~~b)~~ c) a controller in communication with the detection mechanism, wherein the controller is programmed to perform the steps of:
  - i) deriving a growth curve from the measurements of the signal;
  - ii) calculating a derivative of the growth curve;
  - iii) identifying a characteristic of the derivative; and
  - iv) determining the threshold cycle number associated with the characteristic of the derivative.

Claim 2 (Original): The apparatus of claim 1, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

Claim 3 (Original): The apparatus of claim 1, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a negative peak of the second derivative.

Claim 4 (Original): The apparatus of claim 1, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.

Claim 5 (Original): The apparatus of claim 1, wherein the controller is programmed to calculate a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

Claim 6 (Previously presented): The apparatus of claim 1, wherein the controller is programmed to calculate second derivative values of the growth curve at a number of different cycles in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the controller is further programmed to determine the threshold cycle number associated with the positive peak by:

- i) fitting a second order curve to the second derivative data points; and
- ii) calculating the threshold cycle number as the location, in cycles, of a peak of the second order curve.

Claims 7-49 (canceled).

Claim 50 (Currently amended): An apparatus for determining a threshold cycle number in a nucleic acid amplification reaction, the apparatus comprising:

- a) a thermal cycler suitable for a nucleic acid amplification reaction;
- b) at least one detection mechanism for measuring, at a plurality of different times during the amplification reaction, at least one signal whose intensity is related to the quantity of a nucleic acid sequence being amplified in the reaction; and
- b) a controller in communication with the detection mechanism and thermal cycler, wherein the controller is programmed to perform the steps of:
  - i) storing signal values defining a growth curve for the nucleic acid sequence, wherein the growth curve expresses signal intensity as a function of cycle number in the reaction;
  - ii) determining a derivative of the growth curve, wherein the derivative is determined with respect to cycle number; and

- iii) calculating the threshold cycle number associated with a characteristic of the derivative.

Claim 51 (Previously presented): The apparatus of claim 50, wherein the controller is further programmed to identify the characteristic of the derivative as the amplification reaction is occurring and to terminate the amplification reaction when the characteristic is identified.

Claim 52 (Previously presented): The apparatus of claim 50, wherein the controller is programmed to determine the second derivative of the growth curve, and wherein the threshold cycle number is calculated as the location, in cycles, of a maximum of the second derivative.

Claim 53 (Previously presented): The apparatus of claim 50, wherein the controller is programmed to determine the second derivative of the growth curve, and wherein the threshold cycle number is calculated as the location, in cycles, of a minimum of the second derivative.

Claim 54 (Previously presented): The apparatus of claim 50, wherein the controller is programmed to determine the second derivative of the growth curve, and wherein the threshold cycle number is calculated as the location, in cycles, of a zero-crossing of the second derivative.

Claim 55 (Previously presented): The apparatus of claim 50, wherein the controller is programmed to determine the first derivative of the growth curve, and wherein the threshold cycle number is calculated as the location, in cycles, of a maximum of the first derivative.

Claim 56 (Previously presented): The apparatus of claim 50, wherein the characteristic of the derivative comprises a maximum of the second derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

- calculating second derivative values of the growth curve, with respect to cycle number, at a number of different measurement points to yield a plurality of second derivative data points;

fitting a second curve to at least three of the second derivative data points; and  
calculating the threshold cycle number as the location, in cycles, of a positive peak of the  
second curve.

Claim 57 (Previously presented): The apparatus of claim 56, wherein the threshold cycle number at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.

Claim 58 (Previously presented): The apparatus of claim 50, wherein the characteristic of the derivative comprises a minimum of the second derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

calculating second derivative values of the growth curve, with respect to cycle number, at  
a number of different measurement points to yield a plurality of second derivative  
data points;  
fitting a second curve to at least three of the second derivative data points; and  
calculating the threshold cycle number as the location, in cycles, of a negative peak of the  
second curve.

Claim 59 (Previously presented): The apparatus of claim 58, wherein the threshold cycle number at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.

Claim 60 (Previously presented): The apparatus of claim 50, wherein the characteristic of the derivative comprises a maximum of the first derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

calculating first derivative values of the growth curve, with respect to cycle number, at a  
number of different measurement points to yield a plurality of first derivative data  
points;  
fitting a second curve to at least three of the first derivative data points; and

calculating the threshold cycle number as the location, in cycles, of a peak of the second curve.

Claim 61 (Previously presented): The apparatus of claim 60, wherein the threshold cycle number at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three first derivative data points.

Claim 62 (Previously presented): The apparatus of claim 50, wherein the characteristic of the derivative comprises a zero-crossing of the second derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

calculating second derivative values of the growth curve at a number of different measurement points to yield a plurality of second derivative data points; and  
calculating the threshold cycle number at the zero-crossing by interpolation between at least two of the second derivative data points.

Claim 63 (Previously presented): The apparatus of claim 2, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the positive peak exceeds the noise-based threshold level.

Claim 64 (Previously presented): The apparatus of claim 2, wherein the controller is further programmed to perform the step of determining if the positive peak exceeds a user-defined threshold level.

Claim 65 (Previously presented): The apparatus of claim 2, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 66 (Previously presented): The apparatus of claim 2, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 67 (Previously presented): The apparatus of claim 3, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 68 (Previously presented): The apparatus of claim 3, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 69 (Previously presented): The apparatus of claim 4, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 70 (Previously presented): The apparatus of claim 4, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 71 (Previously presented): The apparatus of claim 5, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the positive peak exceeds the noise-based threshold level.

Claim 72 (Previously presented): The apparatus of claim 5, wherein the controller is further programmed to perform the step of determining if the positive peak exceeds a user-defined threshold level.

Claim 73 (Previously presented): The apparatus of claim 5, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 74 (Previously presented): The apparatus of claim 5, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 75 (Previously presented): The apparatus of claim 6, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the peak of the second order curve exceeds the noise-based threshold level.

Claim 76 (Previously presented): The apparatus of claim 6, wherein the controller is further programmed to perform the step of determining if the peak of the second order curve exceeds a user-defined threshold level.

Claim 77 (Previously presented): The apparatus of claim 6, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 78 (Previously presented): The apparatus of claim 6, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 79 (Previously presented): The apparatus of claim 52, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the maximum of the second derivative exceeds the noise-based threshold level.

Claim 80 (Previously presented): The apparatus of claim 52, wherein the controller is further programmed to perform the step of determining if the maximum of the second derivative exceeds a user-defined threshold level.

Claim 81 (Previously presented): The apparatus of claim 52, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 82 (Previously presented): The apparatus of claim 52, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 83 (Previously presented): The apparatus of claim 53, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 84 (Previously presented): The apparatus of claim 53, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 85 (Previously presented): The apparatus of claim 54, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 86 (Previously presented): The apparatus of claim 54, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.



Claim 87 (Previously presented): The apparatus of claim 55, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the maximum of the first derivative exceeds the noise-based threshold level.

Claim 88 (Previously presented): The apparatus of claim 55, wherein the controller is further programmed to perform the step of determining if the maximum of the first derivative exceeds a user-defined threshold level.

Claim 89 (Previously presented): The apparatus of claim 55, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 90 (Previously presented): The apparatus of claim 55, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.

Claim 91 (Previously presented): The apparatus of claim 56, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the peak of the second curve exceeds the noise-based threshold level.

Claim 92 (Previously presented): The apparatus of claim 56, wherein the controller is further programmed to perform the step of determining if the peak of the second curve exceeds a user-defined threshold level.

Claim 93 (Previously presented): The apparatus of claim 56, wherein the controller is further programmed to perform the steps of calculating a noise-based threshold level and determining if the growth curve exceeds the noise-based threshold level at the threshold cycle number.

Claim 94 (Previously presented): The apparatus of claim 56, wherein the controller is further programmed to perform the step of determining if the growth curve exceeds a user-defined threshold level at the threshold cycle number.